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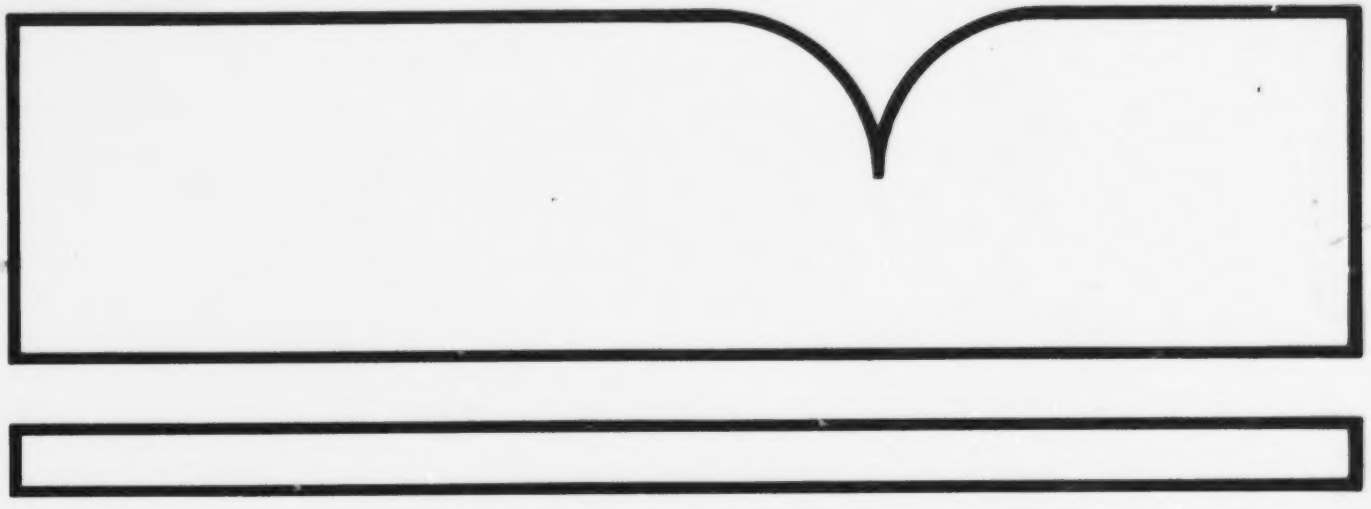

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16. ABSTRACT

The pollution ecology of penaeid shrimps involving industrial organic chemicals, pesticides, petroleum, heavy metals, biological agents, and interactions of the above is discussed. Penaeid shrimps, within their geographic distribution, can serve as valid indicators of the presence and effects of many pollutant agents. Of critical importance for future studies are the interactions of pollutants, pollutant mixes, and physical factors in the environment. In certain specific cases, penaeid shrimps may not be as valuable as other potential indicator species. The value, however, of the penaeid shrimp fishery and their role in the food web of coastal estuarine systems demands that they be studied in relation to human pollution activity in the future.

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CHAPTER 7

Shrimps (Arthropoda: Crustacea: Penaeidae)

JOHN A. COUCH

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I. Introduction

Penaeid shrimps play a significant role in the middle of the trophic web in the estuaries and inner oceanic littoral zones of most temperate, subtropical, and tropical regions of the world. These shrimps have relatively complex life cycles that bring metamorphic stages into contact with a wide range of environmental conditions. If we select as a representative example of penaeid life cycles a species of *Penaeus* in the northern Gulf of Mexico, then the varied environments required are well illustrated in Fig. 1. Gravid females spawn offshore, and early metamorphosis (nauplii, protozoea, and mysis stages) occurs in high-salinity coastal or offshore waters. Postlarvae enter estuaries, become juveniles, and then young adults prior to returning offshore upon sexual maturity (Williams, 1965). The juvenile and young shrimp are intimately dependent upon the quality of estuarine environments. The adults and larvae are dependent indirectly upon quality of the estuaries and directly upon the quality of coastal and near-shore oceanic waters.

Shrimps of the family Penaeidae form the basis for one of the most valuable fisheries in the world. The highly esteemed quality and edibility of these

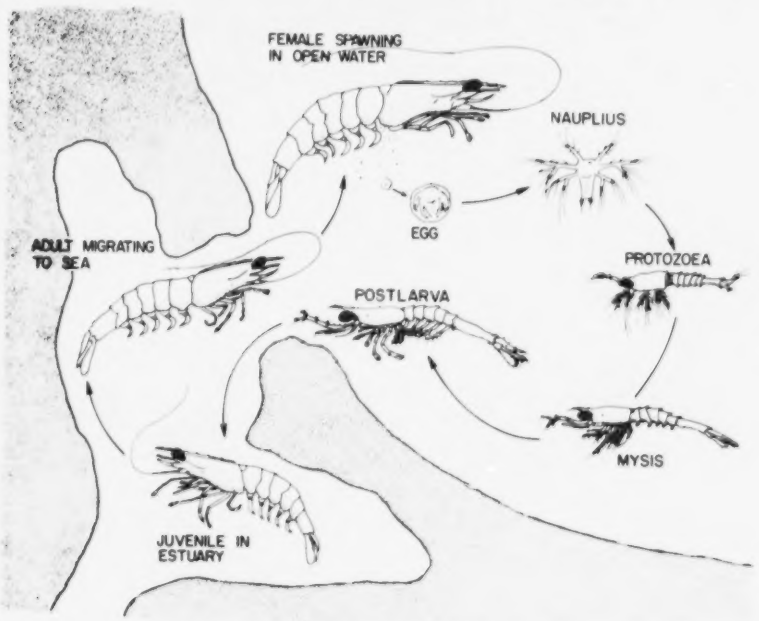

Fig. 1. Life cycle of the penaeid shrimp.

shrimps, their relatively large size, and their broad distribution worldwide have elicited a large investment of manpower and funds in the shrimp industry. In the western hemisphere, the penaeid shrimp fishery is an annual multimillion dollar enterprise; no accurate monetary value can be placed on its worth worldwide.

The purpose of this chapter is to review information available on the pollution ecology of representative species of penaeid shrimps. Data and information from both field and laboratory studies, as reported in the literature and from the author's research, will be used.

The majority of reports available about pollution and penaeid shrimps concerns studies involving the commercially valuable penaeid shrimps of the U.S. Atlantic states and Gulf Coast. Therefore, most of the information presented here will be related to the following three species: *Penaeus duorarum* (pink shrimp), *Penaeus aztecus* (brown shrimp), and *Penaeus setiferus* (white shrimp), all Atlantic and Gulf of Mexico species. Reference to other species of penaeid and some nonpenaeid crustacea will be made when specific studies contribute significantly to our understanding of pollution ecology of shrimps.

This chapter will cover the following pollutant categories and situations: organic chemicals other than petroleum, petroleum and related compounds, heavy metals, biological agents, and interactions of pollutants and other factors. Under each of these divisions toxicity and specific tissue, organismic, population, and ecological effects will be reviewed when known. Further, the uptake, transport, and fate of pollutants will be discussed as they may affect the ecology of penaeid shrimps.

II. Organic Chemicals Other Than Petroleum

A. Industrial Organic Chemicals

The last four decades have witnessed unprecedented release of synthetic organic chemicals into the natural environment. These so-called xenobiotics have numerous origins and uses; rarely are they innocuous upon entering natural waters. Because penaeid shrimps occupy estuaries during a significant portion of their life cycles, they are exposed to pollutants characteristic of industries associated with the estuaries. Relatively few industrial pollutants have been investigated specifically with regard to ecology of penaeid shrimps.

PCB's

Polychlorinated biphenyls (PCB's) are industrial pollutants to be considered with regard to their exemplary roles in penaeid shrimp ecology and aquatic ecology in general. Table I presents a summary of relationships of PCB's and penaeid shrimps.

TABLE I

POLYCHLORINATED BIPHENYLS AND PENAEID SHRIMPS

Compound	Toxicity	Physiological/histological effects	Ecological/environmental implications	References
Aroclor 1254	3 μg/liter 30-day exposure caused 50% mortality, pink shrimp	Lethargy; chromatophore changes; ultrastructural pathology in hepatopancreas cells.	Pink shrimp absorbed 1254 from sediments from polluted estuary; loss of burrowing activity–exposure to predators; shrimp could not avoid contaminated waters as some fish could. Long life of PCB's in sediments maintain pollution at chronic levels over several years.	Duke *et al.* (1970); Nimmo *et al.* (1971, 1975)
	0.9 μg/liter caused some mortality in 14 days, pink shrimp			Couch *et al.* (1974)
Aroclor 1016	10 μg/liter, 96-hour exposure caused 43% mortality	None reported	None reported	Hansen *et al.* (1974)
	0.9 μg/liter, 96-hour exposure caused 8% mortality, brown shrimp			

For many years these industrial chemicals have been at large in the aquatic environment as a result of waste effluents, disposal of dielectric fluids, and other industrial sources (Broadhurst, 1972). It is a well-established fact that certain fresh and marine bodies of water are contaminated with various compounds of PCB (Sodergren *et al.*, 1972; Nimmo *et al.*, 1971, 1975). In 1970, Duke *et al.* reported the PCB, Aroclor 1254*, in water, sediments, and tissue of animals (including penaeid shrimps) from Escambia Bay, near Pensacola, Florida.

Considerable research has been done on the effects of PCB's on estuarine species with emphasis on pink and brown shrimps. These two penaeids were killed in 2-week exposures to 0.9, 1.4, and 4.0 μg/liter Aroclor 1254 in flowing seawater (Nimmo *et al.*, U.S. Environmental Protection Agency, Gulf Breeze, Florida). The minimum concentration causing mortality was 0.9 μg/liter. Penaeid shrimps suffered greatest mortality when exposed during premolt (just before molting) and during molt. Most exposed shrimp became lethargic, stopped feeding, and did not dig into the substrate (digging is a normal activity for penaeids). Subtle to dramatic chromatophore changes in the cuticle of exposed shrimp were more frequent and obvious than in control shrimp.

On the light microscopical level, no lesions were consistently found that were indicative of PCB exposure in shrimp (Couch and Nimmo, 1974a). However, several interesting cytopathic changes were noted in exposed shrimp studied with electron microscopy.

Pink shrimp were exposed to 3 μg/liter Aroclor 1254 in flowing seawater for 30 to 52 days. During these exposures up to 50% of the animals died. Both living and dead shrimp were analyzed by gas chromatography and from 33 to 40 μg/gm Aroclor 1254 was found in their hepatopancreatic tissues. Aroclor uptake in hepatopancreas was linear with time (Couch and Nimmo, 1974a). Hepatopancreas was fixed and processed for electron microscopy. Hepatopancreatic absorptive cells from exposed shrimp revealed the following departures from those of controls: (1) an increase or proliferation in rough endoplasmic reticulum in 30–50% of cells; (2) production of membrane whorls with enclosed lipid droplets; and (3) nuclear degeneration characterized by the occurrence of vesicles in the nucleoplasm (20–50 nm and 100–700 nm in diameter).

The proliferation of smooth endoplasmic reticulum (ER) in hepatocytes of higher animals has been described as indicative of toxic responses to drugs or chemicals such as phenobarbitol, dilantin, dieldrin, and carbon tetrachloride. Proliferation of ER has been related to detoxification of poisons and may, in shrimp, represent an attempt, on the part of hepatopancreatic cells, to metabolize PCB absorbed from the lumen of hepatopancreatic ducts. If this is the case, cellular alterations at the ultrastructural level such as proliferation of ER may be

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valuable as early indicators of sublethal effects of certain pollutants in penaeid shrimps.

Another PCB, Aroclor 1016, has been more recently introduced for limited use in the United States. This compound has been tested for toxicity in brown shrimp. Aroclor 1016 was found to have nearly the same toxicity for penaeid shrimp as Aroclor 1254: 0.9 μg/liter Aroclor 1016 in flowing seawater killed 8% of test shrimp in 96 hours; 10 μg/liter Aroclor 1016 killed 43% of test shrimp in 96 hours (Hansen *et al.*, 1974a).

It is apparent that PCB's as pollutants pose a threat to penaeid shrimps which show a high sensitivity to these compounds. In this regard, Nimmo *et al.* (1971) demonstrated that pink shrimp could absorb a PCB (Aroclor 1254) from sediments taken from a PCB-polluted estuary, Escambia Bay, Florida. Hansen *et al.* (1974b) found that some estuarine species could avoid waters contaminated with Aroclor 1254, but pink shrimp showed no avoidance reaction when given choices of clean or PCB-contaminated water. These and other data suggest that PCB's, as pollutants, could have influence on relative survival and abundance of penaeid shrimps in natural waters.

B. Pesticidal Chemicals

In the last 30 years, many kinds of chemical pesticides have been inadvertently or intentionally released into the environment. Aquatic life is exposed to these compounds because the aquatic portion of the biosphere often behaves as a "sink" or receptacle for these compounds because of runoff or fallout. Some of these pesticides, such as certain organochlorines or their metabolites, are refractory to breakdown, and thus tend to accumulate in various compartments of the aquatic environment. Shrimps have been found to accumulate certain pesticidal compounds in the laboratory, and feral shrimps have possessed detectable levels when taken directly from contaminated or apparently "clean" waters. The staff of the U. S. Environmental Protection Agency Laboratory, Gulf Breeze, Florida, has found, over several years of testing, that penaeid shrimps generally are far more sensitive to toxic and ecological effects of most pesticides than are fishes or mollusks (Couch, 1978). The effects of some of the well-known compounds will be reviewed here.

1. Organochlorines

The following organochlorine pesticides have been studied in regard to organismic and/or ecological effects on penaeid shrimps: chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, lindame, mirex, and toxaphene. Data on the effects are summarized in Table II for these pesticides.

White shrimp, which died as a result of DDT exposure, accumulated up to 40.4 μg/gm DDT and DDE in hepatopancreas after 18 days of exposure to 0.20

TABLE II

ORGANOCHLORINE PESTICIDES AND PENAEID SHRIMPS

Pesticide	Toxicity	Physiological/histological effects	Ecological implications	References
Chlordane	96-hour LC_{50}, 0.4 μg/liter, pink shrimp	None reported	Residues in estuaries from five southeastern states; effect in nature unknown	Parrish *et al.* (1976)
DDT	18 days LC_{100}, 0.1 μg/liter, white shrimp	Loss of cations (Na^+, K^+) in hepatopancreas, possible ATPase inhibitor	Death of exposed shrimp takes out possible step in trophic accumulation in nature	Butler *et al.* (1970), Nimmo *et al.* (1970)
Dieldrin	96-hour LC_{50}, 0.9 μg/liter, pink shrimp	None reported	Commonly found in estuaries; second most common pesticide in mollusks (1965–1972)	Parrish *et al.* (1973), Butler (1973)
Endrin	96-hour LC_{50}, 0.28 μg/liter, pink shrimp	None reported	None reported	Schimmel *et al.* (1974)
Heptachlor	96-hour LC_{50}, 0.11 μg/liter, pink shrimp	None reported	None reported	Schimmel *et al.* (1976)
Hexachlorobenzene	Lethargy in 25 μg/liter and 33% death at 96 hours, pink shrimp	Hepatopancreas grossly abnormally white	Accumulated by estuarine fishes. Exposed shrimp possibly unable to escape predation due to lethargy	Parrish *et al.* (1974)
Lindane	24–96 hour LC_{50}; range, 0.17–35 μg/liter; pink, brown, white shrimps	None reported	None reported	Chin and Allen (1957), Schimmel *et al.* (1977)
Mirex	7-day LC_{25}, 1.0 μg/liter, pink shrimp	Delayed toxicity to pink shrimp 4 days after removal from mirex	Mirex applied as a particle bait in nature may render it unavailable to many organisms	Lowe *et al.* (1971), Tagatz *et al.* (1976), Markin *et al.* (1974)
Toxaphene	LC_{50} nauplius, 22 μg/liter; LC_{50} protozoea, 1.8 μg/liter; LC_{50} mysis, 1.4 μg/liter, pink shrimp	Differential toxic responses of different larval stages	Temperature dissolved O_2 variations affected survival in exposed mysis	Courtenay and Roberts (1973)

μg/liter in flowing seawater (Nimmo *et al.*, 1970). Exposure to DDT concentrations greater than 0.10 μg/liter was lethal to pink shrimp in 28 days. A physiological effect of DDT exposure in pink and brown shrimp was loss of certain cations in the hepatopancreas (Nimmo and Blackman, 1972). Sodium and potassium concentrations in shrimp exposed to 0.05 μg/liter DDT for 20 days were lower than in those not exposed. Magnesium, however, was not significantly lowered. The significance of reduced cations in the hepatopancreas of shrimp for the pathophysiological behavior of shrimp is not known but a loss of ATPase activity in ion transport may be indicated. Blood protein levels also have been found to drop in shrimp exposed to DDT. There are no reports of histopathological changes in penaeids following exposure to DDT. In acute, high-concentration laboratory exposures, shrimp showed tremors, hyperkinetic behavior, and paralysis, classic signs of DDT poisoning in arthropods. After extended exposure to low concentrations of DDT, shrimp did not become paralyzed but sank into lethargy, refused food, and then died.

Pink shrimp were more sensitive to dieldrin than were grass shrimp (Palaemonidae) in test exposures. However, both species died when exposed to concentrations of dieldrin in the low parts per billion range.

Some juvenile pink and brown shrimp died after laboratory exposure to low concentrations of mirex (Table II). However, all survivors from this test died after 4 days in mirex-free seawater, demonstrating a delayed toxic effect of mirex (Lowe *et al.*, 1971). Mirex poisoning in shrimp produces loss of coordination, equilibrium, and finally signs of lethargy and paralysis.

I have examined both shrimp and blue crabs exposed to low concentrations of mirex for long periods (30 days or more) for histopathological effects. No pathologic effects at the tissue level were found in the animals that were examined. The organs studied were muscle, hepatopancreas, and gonads.

Markin *et al.* (1974) completed a detailed ecological monitoring study of the distribution of mirex in oysters, crabs, shrimps, and fishes from estuarine waters from the Gulf of Mexico to Delaware Bay. Sampling stations were in Mississippi Sound; Mobile Bay, Alabama; Tampa Bay, Florida; Jacksonville, Florida; Savannah, Georgia; Charleston, South Carolina; Morehead City, North Carolina; Chesapeake Bay; and Delaware Bay. Only shrimp from the Savannah, Georgia area had detectable concentrations of mirex (0.007 μg/gm in tissues). The Savannah, Georgia area has a long history of mirex usage. Mirex does not appear to be as widespread in estuarine regions as are PCB's and DDT (Markin *et al.*, 1974). Mirex applied usually as a particle bait poison would not be as directly available to many marine organisms as are broadcast liquids or powder formulations of other pesticides.

The finding of 0.007 μg/gm mirex in shrimp near Savannah probably reflects a considerable accumulation of mirex by shrimp over a long period of time from possibly extremely low concentrations in the environment. Lowe *et al.* (1971)

found (Table II) that 25% of test pink shrimp died in the laboratory after 7 days of exposure to only 1.0 μg/liter mirex. Therefore, the concentrations of mirex must be assumed to be considerably lower than 1.0 μg/liter in Georgia estuarine waters. If the aqueous concentrations were above 1.0 μg/liter few shrimp would probably survive in that area.

Toxaphene affects differentially the early metamorphic stages of pink shrimp (Table II) according to Courtenay and Roberts (1973). They found the mysis stage of pink shrimp most susceptible to toxaphene, particularly under various temperature and dissolved oxygen conditions. This might suggest that fluctuations in abundance in juvenile and young adult penaeid shrimps in toxaphene contaminated waters could result from impact of the pesticide on an early life-cycle stage during periods of changing temperature and/or during periods of dissolved oxygen fluctuations.

2. *Organophosphates and Carbamates*

Few organophosphate compounds have been tested on species of crustaceans. However, those tested have shown approximately 1000 times greater toxicity to shrimps than most other pesticides tested (Butler, 1966), and penaeid shrimps have shown greater sensitivity than fishes or mollusks to organophosphates.

Baytex (Bayer 29, 493) was toxic to penaeid shrimp (Butler and Springer, 1963) in the laboratory. Naled (1,2-dibromo-2,2-dichlroethyldimethyl phosphate) had little effect in field tests on shrimp. Fast dilution and instability without persistence of compounds may contribute to the lack of mortality of shrimps in field testing of this organophosphate. In the laboratory, Dibrom is lethal to postlarval brown shrimp at 2.0 μg/liter, and at 5.5 μg/liter (= LC_{50} for 48 hours) it is lethal to adult pink shrimp. Malathion at 14 μg/liter caused hyperactivity, paralysis, and death in penaeids. Parathion lethal concentration for 48 hours in pink shrimp was 0.2 μg/liter (D. Coppage, personal communication). No histopathogenesis has been reported for penaeids exposed to organophosphates.

Conte and Parker (1975) found malathion aerially applied to flooded marshes in Texas caused from 14 to 80% mortality in brown and white shrimps held in cages. They recommended that malathion not be applied to flooded marshes that maintained shrimp. Tagatz *et al.* (1974), however, found that caged pink shrimp were not killed in small areas receiving malathion via thermal fogging for mosquito control in salt marshes in northwest Florida.

Both organophosphates and carbamates are reported to be potent acetylcholinesterase (AChe) inhibitors in the vertebrates. Little evidence of early, presyndromic inhibition of AChe activity in ventral nerve cord of pink shrimp was found, but inhibition as high as 75% was found in moribund shrimp experimentally exposed to malathion (Coppage and Matthews, 1974).

Carbamate pesticides have not been tested extensively in regard to penaeid shrimps, but sevin may be lethal to other crustacea when applied to field sites in the marine environment (Haven *et al.*, 1966). J. Lowe (personal communication) has found carbaryl (sevin) to be quite toxic to penaeid shrimps in laboratory tests. Tagatz *et al.* (1979) report that brown shrimp have a 24- to 96-hour LC_{50} of 2.5 μg/l, the lowest concentration of sevin reported to kill any crustacean tested.

III. Petroleum

Charter *et al.* (1973) estimated that the annual spillage of oil and oil products into the ocean is approximately 3 million tons. Most of these spills occur in estuaries or near coastal regions of significant biological value. Of particular importance is the fact that many coastal areas impacted by oil spills or which are potential spill areas are also in penaeid shrimp-producing regions. Unfortunately, only a few detailed studies of ecological and physiological effects of oil on penaeid shrimps have been published.

Neff *et al.* (1976a) found that when penaeid shrimps were experimentally exposed to oil-contaminated seawater, they accumulated hydrocarbons in their tissues. Table III gives information on two forms of water-soluble petroleum products in relation to penaeid shrimps. Aromatic hydrocarbons were accumulated in greater amounts than were alkane hydrocarbons. Bioaccumulation of aromatics increased in proportion to the increase in their molecular weights up to, but not including, the heavier polycyclic aromatics (four to five rings). Brown shrimp (*Penaeus aztecus*) exposed to oil in seawater and then depurated in clean seawater released accumulated hydrocarbons more rapidly than clams (*Rangia cuneata*) and oysters (*Crassostrea virginica*). Shrimp can metabolize the hydrocarbons whereas the mollusks have limited, if any, capacity to do this. The higher molecular weight hydrocarbons are released from shrimp tissues more slowly than lower molecular weight hydrocarbons (Neff *et al.*, 1976a). Carcinogenic polycyclic hydrocarbons such as benzo[*a*]pyrene, therefore, would be retained longer, possibly to exert chronic effects or to be shunted into the human food chain.

Anderson *et al.* (1974a) tested water-soluble fractions and oil in water dispersions of four oils [(South Louisiana crude; Kuwait crude; refined No. 2 fuel; and Bunker C (residual)] against brown shrimp postlarvae for toxicity. The fractions of refined oils were generally more toxic to penaeid shrimps and to other aquatic species tested than were the fractions of crude oils. Crustacean species tested, including brown shrimp postlarvae, were more sensitive to oil fractions than fish species tested (*Cyprinodon, Menidia,* and *Fundulus*). The 24-hour median tolerance limit of juvenile brown shrimp exposed to components of No. 2 fuel oil (naphthalenes, methyl naphthalenes, and dimethyl naphthalenes) ranged from 0.77 to 2.51 ml/liter. The naphthalenes were the most toxic components of fuel oils (Anderson *et al.*, 1974b) to shrimp.

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TABLE III

PETROLEUM AND PENAEID SHRIMPS

Component	Toxicity	Physiological implications	Ecological/environmental implications	References
Water-soluble fraction of No. 2 fuel oil	2.9 mg/liter = 96-hour LC_{50}, brown shrimp juveniles; 1.0 mg/liter = 96-hour LC_{50}, white shrimp juveniles	Differential susceptibilities of postlarvae, early juveniles, and late juveniles of single species, i.e., brown shrimp. Postlarvae are more tolerant than juveniles	Different susceptibilities of different species in same genus, i.e., brown shrimp vs. white shrimp; might lead to changes in species composition in polluted regions	Neff *et al.* (1976)
Louisiana crude oil (water-soluble fraction)	19.8 mg/liter = 96-hour LC_{50}, brown shrimp postlarvae	May show chronic toxicity, but not as acutely toxic as refined product above	Brown shrimp postlarvae less sensitive than polychaetes and one fish (*Menidia* sp.); about equal in sensitivity with two fishes (*Fundulus* and *Cyprinodon*)	Neff *et al.* (1976)

Steed and Copeland (1967) and Neff *et al.* (1976b) observed considerable interspecies variation in oxygen consumption between different species of *Penaeus* and among different genera of marine animals when they were exposed to the same oil–seawater mixtures. Therefore, physiological responses to oil pollution may vary with test species used, and one cannot with certainty predict a priori which species will be more sensitive to oil (Neff *et al.*, 1976b).

Studies of detailed pathologic mechanisms of oil toxicity in shrimps have been reported only rarely. Yarbrough and Minchew (1975) reported nonspecific lesions in the cuticular chitin, the lining of the gastric mill, and the mouth region of penaeid shrimp exposed to sonified crude oil. They also found the proliferation of cells and necrosis in the basal portion of gill filaments of exposed shrimps.

The ecological effects of an oil spill depend to a large extent upon the environmental, meterological, and geographical variables surrounding the spill. Oil retained in enclosed coastal regions might be expected to affect juvenile shrimps more seriously than would spills occurring in open ocean areas. Oil spills in nature have not been studied specifically in relation to penaeid shrimp effects.

IV. Heavy Metals

A variety of heavy metals are found as both naturally and anthropogenically derived components of the estuarine environment. These metals may exist in several oxidation states with different reaction potentials depending on their specific chemistries (Wood, 1974). Certain heavy metals are pollutants generated by industry. Some may be acted upon by estuarine microbes to produce alkyl-metallic compounds which can be accumulated by estuarine species and are potent toxicants.

The heavy metals that can be methylated, such as mercury, tin, palladium, platinum, gold, and thallium pose special threats as environmental pollutants and some should be monitored continuously (Wood, 1974). Other metals such as cadmium, lead, and zinc do not form stable alkyl-metals in aqueous solutions, but may have different modes of toxic action than do the alkyl-metals such as methylmercury, a neurotoxicant. Cadmium, which does not persist as an alkyl-metal in aquatic systems, but does as an ion, is a strong cytotoxicant to gill cells of crustacea (see below). Reports on effects of cadmium, copper, and mercury have been published concerning penaeid shrimps, and are reviewed below (see also Table IV).

A. Cadmium

Unusually high levels of cadmium have been reported from certain estuaries in which penaeid shrimps commonly occur (i.e., Laguna Madre, Corpus Christi,

TABLE IV

HEAVY METALS AND PENAEID SHRIMPS

Metal	Biologically active state	Toxicity	Physiological/histological effects	Environmental implications	References
Cadmium	Ca^{2+}	718 μg/liter = 30-day LC_{50} pink shrimp	Destroys gill respiratory, osmoregulatory tissues. Causes black gills	Salinity, respiratory stresses combined with cadmium toxicity causes mortality of shrimp	Couch (1977), Nimmo *et al.* (1977)
Copper	Cu^{2+}	0.05 mg/liter, lethal to larval stages of brown shrimp in seawater brine mixture	None reported	Copper tubing used in desalination plants may contribute copper to estuaries	Mandelli (1971)
Mercury	$Hg\text{-}CH_3$, Hg^{2+}	17 μg/liter = 96-hour LC_{50} for postlarval white shrimp	Causes inhibition of brown shrimp's ability to regulate internal ion concentrations. Dysfunction in osmoregulation under salinity stress		Petrocelli *et al.* (1975), Green *et al.* (1976)

Texas). This metal is also a pollutant from several industrial effluents into aquatic systems.

Nimmo *et al.* (1977) observed that in a significant number of pink shrimp exposed to approximately 760 μg/liter cadmium (as $CdCl_2$) for 9 days or longer in flowing seawater, an unusual blackening of gills occurred. Control shrimp did not develop black gills. It was found that the LC_{50} of cadmium in 30 days was 718 μg/liter, and that during these tests many exposed shrimp developed the black gill syndrome prior to death.

I have completed light and electron microscopic studies of gill tissues from exposed blackened gills and control gills of surviving pink shrimp from the above tests (Couch, 1977). My findings indicate that the gross blackening of gills results from necrosis of subcuticular tissues (gill epithelial tissue). This necrosis stems from the death of cells in the distal gill filaments (smallest unit in gill of shrimp). Actual cell death occurs prior to gross blackening in tiny foci, followed by gradual involvement of the whole filament. Electron microscopy reveals polymorphic black deposits in the cytoplasm of moribund or necrotic gill cells (early around mitochondria, later throughout). A complete loss of structural and concomitant functional integrity of the gill soft tissue leads to organ necrosis. However, the cuticle and epicuticle remain intact at the ultrastructural level and hold the moribund or necrotic soft tissue within their boundaries. Apparent melanization of injured gill filaments may hasten the blackening syndrome. However, electron microscopy does not present evidence for the presence of melanosomes, melanocytes, or melanophores. An alternative possibility, that cell death and necrosis lead to the deposition of metal sulfides or other black deposits in necrotic tissues in the living animal, could account for the blackened gill syndrome. The interesting concept of specific cell and tissue death preceding organismic death is represented in the pink shrimp's response to cadmium exposure. Death of cells concerned with osmoregulation and respiration probably lead to dysfunction and eventual death of shrimp.

Bahner (1975) studied the uptake of cadmium in the tissue of pink shrimp. He found that between 1 and 10 μg/liter Cd in water elicited uptake by hepatopancreas, gills, and exoskeleton. Below concentrations of 1 μg/liter Cd, there was no accumulation of the metal in shrimp tissues.

B. Copper

Mandelli (1971) found that a copper concentration of 0.05 mg/liter was lethal to nauplii, protozoea, and mysis of *Penaeus aztecus* and *P. duorarum* that were exposed in a seawater–brine mixture similar to mixtures derived from desalination plants. The larval stages were able to grow normally in seawater (35‰) containing 0.025 mg/liter copper.

C. Mercury

Mercury as a metal has not been suspect in toxic effects on organisms; however, mercuric salts and methylated mercury, are extremely toxic with both short- and long-term chronic effects. Mercuric chloride is used in some histological fixative fluids because of its protein-precipitating effects in tissues. Enzymes of liver and other tissues may be bound and their activity may be altered by ionic mercury.

Petrocelli *et al.* (1975) studied the uptake and distribution of mercuric chloride in brown shrimp (*P. aztecus*), and also examined the effects of mercuric chloride exposure on ability of brown shrimp to adjust to salinity changes. They found that after 2 hours of exposure to 0.5 μg/liter mercuric chloride in seawater, accumulation of mercury in shrimp was 285 mg/gm with only 9% of the mercury in the meat (muscle) and 91% in the shell. This suggested a surface adsorptive process for mercury in brown shrimp exposed for brief periods. These authors also reported that shrimp obtained off Louisiana's Southwest Pass had natural levels of only 4.6 ng/gm mercury distributed as 64% in the muscle and 36% in the cuticle, suggesting that chronic exposure results in internal accumulation of mercury.

Green *et al.* (1976) found that 17 μg/liter ionic mercury is the LC_{50} (96 hours) for postlarval white shrimp (*P. setiferus*). Chronic exposure of postlarval white shrimp to 1.0 μg/liter for 60 days did not affect respiration, growth, and molting (Green *et al.*, 1976).

Brown shrimp are active regulators of blood chloride levels (ion regulators). Petrocelli *et al.* (1975) found that exposure of brown shrimp to mercury and to salinity changes resulted in interference with the shrimp's ability to regulate their internal ion levels to compensate for external salinity changes. Mercury could prove to be detrimental to penaeid shrimps if the form and amount were sufficient enough to prevent their adjustment to freshets or high saline conditions that result from rapid changes in estuaries or tidelands due to seasonal or meterological extremes.

To date, no reports have been published which suggest a specific mechanism for mercury toxicity in shrimps or other invertebrates. Investigations on the relative roles of different species of certain heavy metals need to be carried out to determine the behavior of specific metals in their different chemical states in estuaries.

V. Biological Agents

This section includes a discussion and review of biological agents which are being considered or developed for commercial, agricultural, and health purposes as biopesticides or biological control agents. Two major categories that may

influence penaeid shrimps are biochemical agents (arthropod hormonal agents or their mimics) and infectious pathogenic agents for arthropods (viruses, bacteria, fungi, protozoa, helminths, and parasitoids).

To date, there is no strong evidence to assign any of the above roles as pollutant factors in penaeid shrimp ecology. There is concern, however, for the future use and safety of such agents in coastal regions because of their specific roles in arthropod ecology, physiology, and pathology (Couch, 1975). Biological control agents must be tested and evaluated regarding their safety for nontarget species such as valuable aquatic arthropoda (i.e., crustacea and some aquatic insecta). The effects of some insect growth regulators and insect pathogens, which are being used or developed as biocontrol agents, have been tested in nontarget crustacean species such as penaeid shrimps or related forms. The results of these studies are reviewed in the following sections.

A. Biochemical Agents

Naturally occurring insecticidal chemicals such as pyrethrum, nicotine, rotenone, hellebore, ryania, and sabadilla will not be discussed here because of lack of evidence that they are potential pollutants (Soloway, 1976). Insect growth regulators, however, have shown promise as synthetic insecticides and have the potential to be used on a wide scale thus resulting in possible environmental exposure of nontarget species.

The major example of an insect growth regulator that has been tested for effects on crustacea is methoprene (Altosid). This compound is the only registered (Environmental Protection Agency) commercially available insect growth regulator, and is listed as a control agent for floodwater mosquitoes. Persistence of methoprene in the aquatic environment is of short duration (4–7 days or less according to Siddall, 1976), and does not bioaccumulate. Methoprene is effective in many different insect species but has not shown significant toxicity to nontarget species (Miura and Takahashi, 1973). The LC_{50} of methoprene for white and pink shrimps (penaeids) was 100 ppm according to Siddall (1976) and Wright (1976). Freshwater crayfish also required relatively high concentrations (LC_{50} = 100 ppm) for toxic effects.

There are no reports available on sublethal physiological effects of an insect growth regulator in penaeid shrimps. Their mechanisms of effect in insects (interference with maturation and reproduction) should be examined as possible mechanisms in Crustacea because of relative close phylogenetic affinities of Crustacea and Insecta.

B. Infectious Pathological Agents

The major group of potential bioinsecticides studied in penaeid shrimps have been the entomopathogenic viruses known as nuclear polyhedrosis viruses

(*Baculovirus* group). These insect viruses are of particular interest because related viruses have recently been found in feral and cultured shrimps (Couch, 1974, 1975, 1978) and in feral crabs (Bazin *et al.*, 1974; Johnson, 1976) as natural pathogens.

Couch (1975), Lightner *et al.* (1973), Summers *et al.* (1975), and Heimpel and Buchanan (1967) have discussed the potential relationships between insect Baculoviruses (being developed as biological control agents) and nontarget species from shrimps to man. Summers *et al.* (1975) has pointed out the need to understand better the physical and biochemical nature of these viruses in different host systems. Couch (1975) suggested a need to evaluate regularly and experimentally new viral insecticides in nontarget crustacean species such as penaeid shrimps, with an emphasis on potentially susceptible larval stages of shrimps. Lightner *et al.* (1973) reported that tests of insect Baculoviruses in penaeid shrimps revealed no susceptibility of shrimps for the then-available insect viral insecticidal formulations. Postlarval, early, and late juvenile stages of brown and white shrimps were inoculated with free virus and were fed a diet containing virus. Over a 30-day test period, no mortality attributable to virus exposure occurred in test shrimps. These types of cross-infection experiments (i.e., insect virus in shrimp) should include careful ultrastructural, biochemical, and genetic monitoring to ensure that sublethal infections establishing the virus in a new host do not occur.

The modes of behavior in the estuary of viral, fungal, bacterial, and protozoan biological control agents developed as commercial products and for use in coastal agriculture are not known. The future need to understand the fates and effects of novel agricultural agents in estuaries is predictable, particularly regarding the development of integrated pest control methods which may utilize combinations of chemical and biological control agents. Mutagens in the form of chemicals such as fertilizer components, pesticides, or preservatives may interact with biological control agents in ways not presently anticipated, resulting in mutants with expansionist potential. Viruses, bacteria, and fungi should be studied in this regard.

VI. Interactions of Pollutants, Pollutant Mixes, and Other Factors in Penaeid Shrimp Ecology

Perhaps the most important area for future research in pollution ecology is the study of interactions of specific pollutants and pollutant complexes with physical, chemical, and biological factors in the environment. Estuaries are prime examples of multivariate and complex ecosystems where no single variable is altered without influencing some other component of the system. Penaeid shrimps are very dependent upon varying physical, chemical, and biological

factors for their survival because of the wide range of factors that they must contend with in their life cycle, ranging from the open ocean to the tidal estuary.

Chief among the natural factors influencing penaeid shrimps are salinity, temperature, oxygen concentration (Venkataramiah *et al.*, 1977), bottom types or substrate, nutrition, and infectious diseases (Couch, 1978). A severe or sudden perturbation of any of these factors, combined with pollutant stress, may affect total stress or injury in penaeid shrimps. Some two-factor interactions will be reviewed.

A. Pollutant-Salinity Stress Interactions

Few reports have been published on the interaction of pollutant and salinity stress in penaeid shrimps. Nimmo and Bahner (1974) found that when brown shrimp were exposed to 3.0 μg/liter of the polychlorinated biphenyl Aroclor 1254 and to a gradual decrease in salinity over an 8-hour period, mortality of exposed shrimp was greater than in controls exposed to salinity change only or PCB only. The 3.0-μg/liter concentration of PCB was found previously to be sublethal in an 8-hour period. Therefore, the deciding factor that induced mortality was the combination of PCB exposure and salinity change. The authors also found that most major ions in the blood of dying shrimp became significantly less as the ambient salinity decreased. However, osmotic pressure overall showed no corresponding loss in the PCB-salinity stressed shrimp, thus complicating any interpretation of physiological mechanism for the lethal effect.

Jones (1975) reported that cadmium, zinc, and lead toxicity was enhanced in marine and estuarine isopods under stressful salinity changes in experimental exposures. Hemolymph osmotic concentrations were altered significantly in isopods exposed to cadmium, zinc, and mercury. Since it is known that cadmium destroys the soft gill tissues of penaeid shrimps (see Section IV and Couch, 1977), it is probable that the ion and water regulatory mechanism at the epithelial and cell membrane levels are structurally and functionally affected in crustacea exposed to certain heavy metals. Thus when salinity changes occur, the osmoregulatory mechanisms in metal-exposed crusacea fail to respond or are inhibited.

B. Pollutant-Temperature Stress Interactions

Penaeid shrimps have definite upper and lower temperature tolerance limits; they are shrimps of the temperate, subtropic, and tropic zones. Below water temperatures of about 5°-8°C penaeid shrimps become listless, and at lower temperatures they die. Above temperatures of 33°-35°C penaeids do not do well. Any pollutant that has a high oxygen demand would probably, at higher water temperatures in warm months, contribute to lower oxygen tension in water and

thus to asphyxiation of penaeids that are sensitive to marginal or low ambient oxygen concentrations. No reports were found of studies explicitly concerned with the interactive effects of pollutants and varying temperature in penaeid shrimps.

The synergistic effects of temperature, salinity, and heavy metals in the fiddler crab *Uca pugilator* are discussed by Vernberg and Vernberg (1972). No three-factor interaction reports involving penaeid shrimps were found in the literature.

C. Pollutant-Pollutant Interactions

Most specific pollutants enter the estuary as components of complex mixtures of pollutant fallout, runoff, or effluents. In many cases estuarine species are exposed to several pollutants at once or to varying concentrations of two or more pollutants. Therefore, it is important to know what the combined and single effects of pollutants may be in estuaries. Bahner and Nimmo (1976) have examined the effects of combinations of toxicants in experimentally exposed pink shrimp (*Penaeus duorarum*). They studied the effects of the following toxicant combinations: cadmium-malathion, cadmium-methoxychlor, and cadmium-methoxychlor-Aroclor 1254. Essentially they found, with their particular mixtures, that the toxicities of the combinations reflected no dramatic interactions when compared to the toxicities of each single component of the mixtures. They stated that the toxicity of each component was independent and additive and no synergism was detected. More studies utilizing a broader range of defined pollutant mixes should be done to explore more fully the possibility of pollutant-pollutant interaction and possible synergism.

D. Pollutant-Natural Disease Interaction

Pollutant-disease interaction is one of the least studied phenomena in estuarine pollution ecology. There are numerous examples in man and other vertebrates of pollutant stress leading to increased susceptibility to natural pathogens such as viruses, bacteria, and noninfectious diseases such as neoplasia and respiratory dysfunction. There is growing evidence that some fish diseases are enhanced by certain pollutants (Couch *et al.*, 1974b).

Penaeid shrimps have a virus disease caused by a *Baculovirus* that is enhanced by a chemical pollutant, Aroclor 1254 (Couch, 1974; Couch and Courtney, 1977). This virus-shrimp system has been studied in order to test its suitability as a model indicator system for determining the degree of influence that sublethal concentrations of pollutant chemicals may have on a natural virus-host complex. The pink shrimp (*P. duorarum*) has proven to be a natural host for the shrimp-specific *Baculovirus* originally described as a result of findings made on experimentally stressed penaeid shrimps (Couch, 1974). A captive population of pink

shrimp with relatively low initial virus prevalence stressed by 1–3 μg/liter Aroclor 1254 (PCB) for up to 30 days experienced a significantly more rapid spread and increased prevalence of the *Baculovirus* than did a control, nonstressed population with equal, initial viral prevalence (Couch and Courtney, 1977). The mortality in the Aroclor–*Baculovirus*-stressed population was greater than in the control population. At the end of the test, prevalence of virus infections in the Aroclor-stressed population was approximately 50% more than in the control population and intensity of individual virus infections was greater in the Aroclor-stressed shrimp.

The ecological implications for such findings are suggestive of phenomena in nature that to date have not been evaluated. If population immunity or resistance to natural pathogens were reduced in dense populations of Crustacea by sublethal exposures to specific pollutants, then natural disease, aided and abetted by human pollutant activity, could cause subtle but high mortality over longer periods of time and gradually contribute to an alteration of the ecological balance among certain susceptible species. Whether or not this happens in nature is unknown, but field studies should be conducted to compare disease prevalences in aquatic species in polluted versus nonpolluted regions.

VII. Discussion

Penaeid shrimps are valuable, desirable component species of estuarine ecosystems. The well-being of penaeids could reflect the general quality of their immediate estuarine or offshore environments. Penaeids, for example, are generally more sensitive to some chemical pesticides than are mollusks or most fishes. The fact that species of penaeids are found world-wide in the temperate, subtropic, and tropic regions indicates that they could be studied in these regions as indicators of estuarine health.

There are several characteristics of penaeids, however, that must be taken into consideration before they are used as choice indicator species in pollutant studies. These characteristics are (1) a limited north–south distribution beyond the temperate zones; (2) open ocean littoral and estuarine habitat requirements of life cycle could confuse identity of source of pollutant effects if population fluctuations were considered as criteria for effect; (3) culture of penaeids is difficult and, therefore, laboratory egg to egg or life cycle studies are difficult; and (4) penaeids are less sensitive to some specific pollutants than are other estuarine fishes and invertebrates.

The need to better understand the interaction of physical, chemical, and biological factors and pollutants in the ecology of penaeid shrimps and other estuarine species should be emphasized. Mortalities of penaeid shrimps and most other crustacean species are rarely detected in nature, except occasionally after-

the-fact, as reflected in poorer commercial catches or in population changes. There is a need to develop monitoring and sentinel methods that will permit early detection of injury or damage to estuarine species or alteration of species composition. Without these early-warning methods little can be done to predict and then ameliorate or control the impact of estuarine pollution short of complete halting of pollutant discharge, which seems unlikely with present technology.

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